#13/Brief
Godwin 12/29/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE HONORABLE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of:
Timo Nils-Erik LÖVGREN et al.
Appln. S.N. 08/487,623
Filed: June 7, 1995
For: BIOSPECIFIC ASSAY METHOD

BRIEF ON APPEAL

Assistant Commissioner
for Patents
Washington, D.C. 20231

December 8, 1997

Sir:

This is an appeal from the decision dated March 19, 1997, of the primary Examiner finally rejecting claims 6, 7, 10, 13 and 16-18 in this application.

(1) REAL PARTY IN INTEREST

The real party in interest is Wallac Oy, Turku, Finland.

(2) RELATED APPEALS AND INTERFERENCES

Appellant is not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS

Claims 6, 7, 10, 13 and 16-18 are pending in this application. Claims 1-5, 8, 9, 11, 12, 14 and 15 have been canceled. Claims 6, 7, 10, 13 and 16-18, as finally rejected, appear in the appendix attached hereto.

(4) STATUS OF AMENDMENTS

No response has been filed and no amendments have been made subsequent to issuance of the final Action.

(5) SUMMARY OF INVENTION

The invention recited in appellants' claims relates to a biospecific assay method in which (1) microparticles coated with a bioaffinity reactant A (e.g., an antibody in a competitive assay) which specifically binds at least one analyte (antigen), (2) a labelled bioaffinity reactant B (antigen) and (3) an analyte (antigen) are reacted to cause the analyte and the labelled bioaffinity reactant B to specifically bind to the microparticles via the bioaffinity reactant A. The invention is the discovery

that by appropriate control of the amount of microparticles and the amount of sample, the concentration of an analyte in a predetermined, clinically relevant range of analyte can be determined by measurement of the signal from a surface of a single microparticle. The amount of microparticles and amount of sample are determined by experimentation using samples having a known concentration of analyte.

Thus, it is described in the present application that:

> "According to the present invention such an amount of sample and microparticles is used in the assay that after binding of the analyte of the sample to the said amount of microparticles, each individual microparticle will emit such a signal strength as to allow the measurement of the analyte concentration in the sample over the whole range of typical analyte concentrations, and the signal strength from each microparticle will be measured separately."

(page 5, lines 17 - 25);

> "The present invention allows the measurement of both the highest and the lowest concentrations of analyte from individual microparticles. The sensitivity of the assay and the measurement range are controlled by the amount of microparticles used in the assay."

(page 6, lines 22 - 27);

> "This still allows the remaining signal strength to be reproducibly measured, by the label technology used, from the surface of individual microparticles, and at the same time,

> the signal strength (due to the specific binding of labelled analyte to the coated microparticle) corresponding to the lowest analyte concentration will not exceed the binding capacity of individual particles. The measuring range is adjusted for different assays by altering the amounts of the microparticles and antibodies used in the assay method in such a manner that measurements can always be taken from individual microparticles."

(page 8, lines 22 - 33);

> "If the sensitivity of the label technology is about 6,000 molecules per one microparticle, and with a signal strength directly related to the concentration of the analyte, it is possible for one microparticle to bind the analyte contained in 1 μl, and the amount of the analyte can be measured."

(page 9, lines 6 - 11); and

> "The amount of the microparticles used in the assay, coated with the analyte-specific antibody or bioaffinity reactant as well as the amount of the analyte per microparticle will be adjusted so that a minimal concentration and volume of the analyte will contain enough analyte for binding to the surface of individual microparticles, and enough for measurement from individual microparticles by means of a labelled specific antibody (labelled bioaffinity reactant) and with the sensitive labelled technology used."

(page 9, lines 21 - 30).

The use of single, or individual, microparticles offers considerable advantages over the measurement of the total signal from a certain amount of microparticles (prior art batch method). If, for example, low analyte concentrations are to be measured, a

decrease of the amount of microparticles used in the batch method will cause a decrease in sensitivity. If, on the other hand, single microparticles are measured as in the present invention, a decreased amount of microparticles will lead to an increase in sensitivity. This increase in sensitivity is seen in the data of Tables 2 and 3, page 15, and in Figures 2 and 3 of the present application.

An essential advantage is achieved by using such an amount of sample and microparticles that measurement can be appropriately made from an individual particle. Dilutions are thus avoided. Furthermore, very small analyte concentrations can be measured on individual microparticles, which has not been possible before appellants' invention.

(6) ISSUES

The issues presented for consideration by the Board are:

(A) whether the claims particularly point out and distinctly claim the subject matter appellants regard as their invention;

(B) whether the specification provides a sufficient enabling disclosure of how to make and how to use the invention under 35 U.S.C. § 112, first paragraph; and

(C) whether the claims are *prima facie* obvious under 35 U.S.C. § 103(a) over Soini et al., U.S. Patent No. 5,028,545, taken alone or in combination with Ekins and Chu, Clin. Chem. 37, 1955 (1991) or Buechler et al., U.S. Patent No. 5,089,391, and in further view of Bush et al., Analytical Biochemistry 202, 146 (1992).

(7) GROUPING OF CLAIMS

The claims stand or fall together.

(8) ARGUMENT

A. The claims particularly point out and distinctly claim the subject matter that the **applicants** regard as their invention.

The claims stand finally rejected as being indefinite under the second paragraph of 35 U.S.C. §112 because, in the Examiner's opinion, the applicants have not claimed what they regard as their

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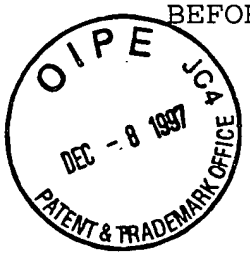

APPEAL BRIEF

Appellant: Timo Nils-Erik LÖVGREN et al.

BIOSPECIFIC ASSAY METHOD

Serial Number: 08/487,623
Filed: June 7, 1995
Appeal No.:
Group Art Unit: 1802
Examiner: C. Spiegel

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A check in the amount of $310.00 is enclosed for the fee for the Appeal Brief. In the event that any additional fees are due with respect to this paper, please charge our Deposit Account No. 11-1833.

Date: December 8, 1997

Atty. Docket No. TUR-026

invention. According to the Examiner, the invention is the discovery that:

> "very low analyte concentrations can be assayed by **decreasing** the amount of microparticle over what is conventionally used in the prior art." (Final Action dated March 19, 1997, page 5, lines 4 - 6, emphasis in the original)

The Examiner is wrong.

First, appellants' invention is not limited to the measurement of low analyte concentrations. It is clear from the portions of the specification disclosure quoted in Section 5 above that appellants' invention allows the measurement of analyte concentrations over "the whole range of typical analyte concentrations" (page 5, lines 23 - 24, of appellants' specification).

Second, although appellants' invention is an improvement in prior art assay methods in that the amount of microparticles required for use in appellants' assay method is typically decreased relative to the amounts of microparticles used in typical prior art assays, decreasing the amount of microparticles relative to the prior art is not appellants' invention. Appellants' invention is the discovery that the analyte concentration in a sample can be

measured from the surface of a single microparticle by adjusting the relative amounts of microparticles and sample. This is the invention that is recited in the claims.

The Examiner also states that:

> "It is unclear whether analyte concentration is measured from a single microparticle measurement *or* an average signal obtained by measuring a predetermined number of individual microparticles is used..." (Final Action, page 5, lines 7 - 10).

This statement is not understood. Claim 13 recites:

> '...measuring the signal strength from an individual microparticle...and determining the analyte concentration in the sample by comparing said signal strength measured from said individual microparticle with a standardization curve,..." (lines 19 - 24).

This language is clear, particularly when read in light of the specification disclosure as it must be. Analyte concentration in a sample is measured using a single microparticle.

The Examiner has also quoted the portion of appellants' response (Paper No. 9 filed December 10, 1996, sentence bridging pages 4 and 5) that states:

"...each of the individual microparticles is not measured..." and appears to suggest that this statement is inconsistent with the invention recited in appellants' claims.

This statement is not inconsistent with appellants' claims. The method of the present invention does not require measurement of each, i.e., **all**, of the individual microparticles used in the assay. The term "each" as used in the context of the quoted statement cannot be interpreted as meaning "a single" (microparticle).

Appellants' claims, therefore, comply with the requirements of the second paragraph of 35 U.S.C. §112. As stated by the Court of Customs and Patent Appeals in the case of In re Borkowski, 164 USPQ 642, 645-46 (1970), quoted with approval in the case of In re Hyatt, 218 USPQ 195, 197 (Fed. Cir. 1983):

> "The first sentence of the second paragraph of §112 is essentially a requirement for precision of claim language. If the scope of subject matter embraced by a claim is clear, and if the appellant has not otherwise indicated that he intends the claim to be of a different scope, then the claim does particularly point out and distinctly claim the subject matter which applicant regards as his invention."

B. The Examiner has failed to provide proper reasoning or evidence to support her position that a person of ordinary skill in the art could not determine analyte concentration based upon the signal from a single, isolated, individual microparticle without undue experimentation.

The Examiner has objected to the specification and has rejected the claims under the first paragraph of 35 U.S.C. §112 for the reason that the specification does not:

> "teach/suggest how to determine analyte concentration based upon the signal from a single, isolated, individual microparticle." (Final Action, page 5, lines 21 and 22)

This rejection is not supported by proper reasoning or evidence.

The specification at page 10, lines 1-5, includes a description of various methods for the measurement of individual microparticles, i.e., flow cytometer, time-resolved microscope or time-resolved microfluorometer or other measuring instruments based on the use of time-resolved technology. U. S. Patent No. 5,028,545 and Seveus L. et al., Cytometry 13: 329-338 (1992) are cited as publications disclosing suitable measuring methods.

The measurement of single microparticles as described in the examples was based on the use of time-result microfluorometry (refer to page 11, lines 12-16, lines 24-26 and lines 31-32, and

page 12, lines 4-6 and lines 32-34 of the specification disclosure). The principle of microfluorometry is, like flow cytometry, well known in the prior art. The microfluorometer apparatus used in the experiments related to this invention is illustrated in Appendix 2 to the response filed June 13, 1995, to the Final Office Action dated December 13, 1994, in parent application S.N. 08/182,550.

In microfluorometry, measurement is carried out on a miniaturized scale using excitation and emission optics that make it possible to read the fluorescence from a very small space such as that occupied by a single microparticle. In the microfluorometer, the fluorescence from a single microparticle at a time is measured. When the fluorescence intensity from a microparticle has been measured, the x-y table can be moved to focus a further microparticle into the reading position. One microparticle at a time is read and the microparticles do not move in any liquid flow during measurement.

Corresponding readings of the fluorescence intensity of individual microparticles can also be measured using a time-

resolved microscope as described in the publication by Seveus et al. cited on a page 10, line 5, of the specification disclosure. In this case, an image of the time-resolved fluorescence of a number of microparticles is taken simultaneously. The result is analyzed with an image analysis program that provides the fluorescence intensity of each individual microparticle. Consequently, a number of microparticles are excited simultaneously and an image of the fluorescence intensity of the microparticles is taken. An image program is used to read the fluorescence intensity from single microparticles and the read value gives the concentration of the analyte measured by that microparticle. No liquid flow or movement of microparticles is required when the time-resolved microscope is used to read the result from single microparticles.

The luminescence signal from an individual microparticle is a measure of the analyte concentration in the sample. As recited in claim 13, the signal measured from an individual microparticle is compared with a standardization curve wherein the standardization

curve is a mean of the signal strength of the predetermined number of uniformly sized microparticles used in the assay method.

The Examiner has not explained why determining analyte concentration based upon the signal from an individual microparticle as described in the application would required undue, or excessive, experimentation and has not otherwise provided evidence or reasoning to support her position.

Reversal of the 35 U.S.C. §112, first paragraph, ground of rejection is in order and is respectfully solicited.

C. The prior art references relied on by the Examiner, taken individually or in any combination, are insufficient to support a case of *prima facie* obviousness of the claims under 35 U.S.C. § 103.

The Examiner has misinterpreted the prior art references in an attempt to reconstruct appellants'invention based on a hindsight analysis in light of appellants' disclosure.

In the Final Action, at page 9, lines 3 - 13, the Examiner has quoted Soini at column 2, lines 20-37. The Examiner's

interpretation of the quoted pertion of Soini is wrong. A proper interpretation is critical to the resolution of the § 103 issue.

Initially, It is noted that appellants' do not dispute that Soini teaches the use of a small sample volume to minimize the time required for equilibrium to be established between the coated microspheres, analyte and labelled reactant. This, however, is not appellants' invention. The primary issue relates to the interpretation of the sentence:

> "A sufficient number of microspheres are analyzed and the fluorescence signals from each microsphere are registered in a computer."

Ekins and Chu has been applied in a way that is not altogether clear to appellants. First, appellants do not understand how the reference can be applied in combination with Soini when the authors admit that the definitions of sensitivity and precision are in dispute. Furthermore, appellants do not believe that the reference can be read other than in terms of the fractional occupancy concept and vigorously assert that the teaching of the reference must include Figure 8 and the explanation at pages 1961 and 1962. Appellants' invention (and that of the Soini reference) is totally

unrelated to the fractional occupancy principle. The teachings of Ekins and Chu and Soini cannot be properly combined and, even if combined, will not result in appellants invention.

Buechler et al. has been cited as a teaching of methods to optimize sampling parameters with particular emphasis upon a labelled ligand analogue. From Figures 4 and 5, it may be seen that the the number of particles which can be used as the terminal solid phase is irrelevant to the analysis. This reference appears to teach away from appellants' invention as disclosed and claimed.

Bush et al. has been applied to claim 7, which is directed the use of the claimed invention for the analysis of nucleic acids by hybridization with a probe. Applicants do not dispute that the reference teaches the use of time-resolved fluorescence as a method for detecting an immobilized nucleic acid (in this case DNA). However, the reference does not teach optimization of the **quantification** of the claimed methodology.

As a final comment regarding the §103 ground of rejection, the Examiner suggests that the present invention is the discovery of "optimum or workable ranges" involving routine skill in the art.

It is well-established, however, that optimization must come from within the teachings of the prior art. The Examiner has failed to cite any art that discloses or suggests that analyte concentration of a sample can be measured from individual microparticles.

For the reasons explained above, none of the cited references, whether considered alone or in combination with the other references or other prior art, discloses or suggests the invention recited in the claims on appeal. The 35 U.S.C. §103 ground of rejection of the claims is improper.

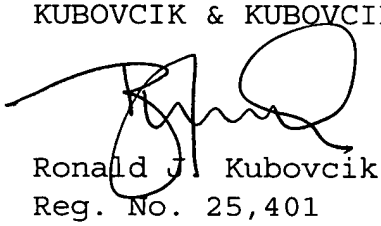
(9) SUMMARY

In view of the foregoing arguments, appellants respectfully submit that each of the rejections made in the Final Rejection is improper. Appellants request that the Final Rejection of the Primary Examiner be reviewed and reversed.

Please charge any required fees or credit any overpayment to our Deposit Account No. 11-1833.

Respectfully submitted,

KUBOVCIK & KUBOVCIK



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APPENDIX

6. The assay method according to claim 13, wherein the assay comprises a non-competitive immunoassay in which the labelled bioaffinity reactant B comprises an antibody which specifically binds to the analyte.

7. The assay method according to claim 13, wherein the assay comprises a nucleic acid hybridization assay, in which the labelled bioaffinity reactant B comprises a nucleic acid probe which specifically hybridizes with the analyte.

10. The assay method according to claim 13, wherein said luminescent label is selected from the group consisting of labels emitting fluorescence, time-resolved fluorescence, chemiluminescence and bioluminescence.

13. In a biospecific assay method comprising

- reacting microparticles coated with a bioaffinity reactant A which specifically binds at least one analyte to be assayed, a sample to be analyzed, and a labelled bioaffinity

reactant B to cause said analyte and said labelled bioaffinity reactant B to specifically bind to said microparticles via the bioaffinity reactant A; and

- measuring signal strength from labelled bioaffinity reactant B bound to the microparticles to determine the analyte concentration in the sample, the improvement comprising:

- contacting a predetermined amount of said sample, a predetermined number of uniformly sized microparticles coated with said bioaffinity reactant A and said labelled bioaffinity reactant B labelled with a luminescent label such that, after the specific binding of the analyte in the sample to said predetermined number of uniformly sized microparticles, each individual microparticle emits a signal strength that corresponds to the analyte concentration in the sample, and

- measuring the signal strength from an individual microparticle using a measuring means capable of reading the luminescence from an individual microparticle and determining the analyte concentration in the sample by comparing said signal strength measured from said individual microparticle with a standardization curve, wherein said standardization curve is a mean

of the signal strength of said predetermined number of uniformly sized microparticles.

16. The assay method according to claim 16, wherein the assay comprises a competitive immunoassay, in which the labelled bioaffinitive reactant B comprises an antigen, and the bioaffinity reactant A is an antibody for whose binding sites the labelled antigen and the analyte compete.

17. The assay method according to claim 6, wherein the amount of said predetermined number of uniformly sized microparticles coated with the antibody A is adjusted so that the lowest analyte concentration will result in the strongest signal.

18. The assay method according to claim 13, wherein the microparticles used comprise a mixture of microparticles recognizing different analytes.